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New dirhodium complex with activity towards colorectal cancer

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ABSTRACT

A novel dirhodium complex (Rh_2(*L*-PheAla)$_2$(OAc)$_2$ is reported with strong activity towards human colon adenocarcinoma cells. Its effect was not accompanied by generation of reactive oxygen species (ROS) neither by activation of caspase-3.

Cisplatin (cis-diammine-dichloroplatinum(II)) was discovered to inhibit *Escherichia coli* cellular division by Rosenberg in 1960s, stimulating the interest by platinum-based compounds.[1] It binds to DNA, preferentially to guanine, to form intrastrand and interstrand adducts.[1,2] However, it presents side effects due to binding to blood plasma proteins leading to kidney and nervous system toxicity, hearing difficulties, nausea and vomiting.[3] Cellular acquired resistance is another problem associated to cisplatin.[4] Consequently, new platinum-based drugs and drugs with different metals as Re, Ru and Rh have been investigated, and combinatory therapies have also been tested.[5] Dirhodium compounds of the type $Rh_2(O_2CR)_4$ L_2 (R = Me, Et, Pr; L = solvent) contain at least two bridging carboxylate ligands and have been shown to possess antitumor activity against Ehrlich ascites and leukemia L1210 tumors, for instance.[6,7] Dirhodium complexes were demonstrated to bind to double stranded DNA and to inhibit DNA replication in vitro.[8] They were also shown to bind directly to T7-RNA polymerase unlike cisplatin and, for some complexes such as Rh_2(*m*-$O_2CCH_3)_4$ and cis-[Rh_2(*m*-$O_2CCH_3)_2$(phen)$_2$]$^{2+}$, it was necessary lesser concentrations to inhibit DNA replication.[8] Dirhodium complexes were shown to bind to nucleobases, dinucleotides and DNA dodecamer single strands.[9] Furthermore, experimental work reported that enzymes with free SH-groups, required for or related to enzymatic activity, were irreversibly inhibited by rhodium (II) carboxylate complexes.[9] This suggests a mode of action different from cisplatin. Cytotoxic activities of such complexes have been related to their molecular characteristics. Permeability is linked to complex lipophilicity and its increase leads to a higher cytotoxicity, except when the carboxylate group goes beyond the pentanoate.[6,10] Liability of the leaving groups of the dirhodium core, presence of open coordination sites, overall charge of the complex and hydrophobicity of the carrier ligands are other factors that affect activity.[11]

The majority of the patients with colorectal cancer are diagnosed with advanced or metastatic disease and just about 10% of them survive[12] Oxaliplatin, 5-fluorouracil, leucovorin, and irinotecan are the current drugs used in chemotherapy.[13] However, they may cause serious side effects as gastrointestinal toxicities and thromboembolic events with consequent treatment discontinuation or patient death.[14] As a result, development of promising anti-cancer candidates with less severe side effects and an increased life expectancy is necessary.

Here is presented newly synthesized dirhodium complexes and their activity on human colon adenocarcinoma cell lines HT-29 and CaCo-2. One of them (**1a**) reduced considerably the growth of HT-29 cells without affecting viability of CaCo-2 monolayer, a good model for toxicity studies.[15] We think the study of complex **1a** should be developed for creation of new opportunities for colorectal cancer treatment. By taking advantage of the possibility to exchange the acetate groups of $Rh_2(OAc)_4$ in water, different α-amino acids were tested. This allowed the formation of new peculiar Rh(II) complexes geometry such as the ones resulted by the coordination of two L-phenylalanine derivatives **1**. The amino

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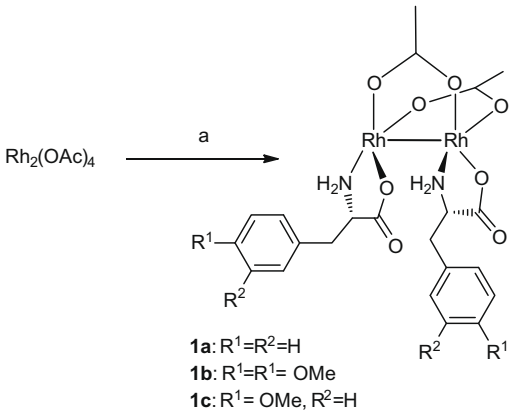
acid units do not maintain the common bridge structure but each amino acid is bound to each rhodium atom (Scheme 1). From different synthesized Rh(II) complexes, derived from phenylalanine (**1a**), 3,4-dimethoxy phenylalanine (**1b**) and methoxy tyrosine (**1c**), **1a** was identified as the best candidate.

CaCo-2 monolayer was treated with different concentrations of each complex and a reference drug, cisplatin. Assessment of viability by a tetrazolium staining shown that none of the tested compounds decreased significantly viability within a period of 4 h, as shown in Figure 1. To evaluate their anti-proliferative activity on the HT-29 cell line, a similar assay was performed. Results were different within the three studied complexes and cisplatin: **1b** and **1c** were unable to reduce proliferation whereas **1a** and cisplatin decreased considerably proliferation within a 24 h period (Fig. 2).

Identical decay of viability was obtained when the exposure time of the complex **1a** and cisplatin was expanded from 4 to 20 h (Fig. 2). Compound **1a** was shown to have a higher activity than cisplatin (Fig. 2).

Levels of reactive oxygen species (ROS) were measured to investigate their involvement in the response induced by compound **1a**. After 2 h treatment with either cisplatin or complex **1a**, cells were washed and treated with a probe, which permeates cell membrane and is oxidized by intracellular ROS turning into a fluorescent molecule. As controls, we have used carrier solvent treated cells. From Figure 3, it is clear an increase in ROS only for cisplatin-treated cells.



Scheme 1. Preparation of new Rh_2(*L*-PheAla derivative)$_2$(OAc)$_2$ **1** complex in water from $Rh_2(OAc)_4$. Reagents and conditions: (a) amino acid (5 equiv) H_2O, 80 °C, 56 h.

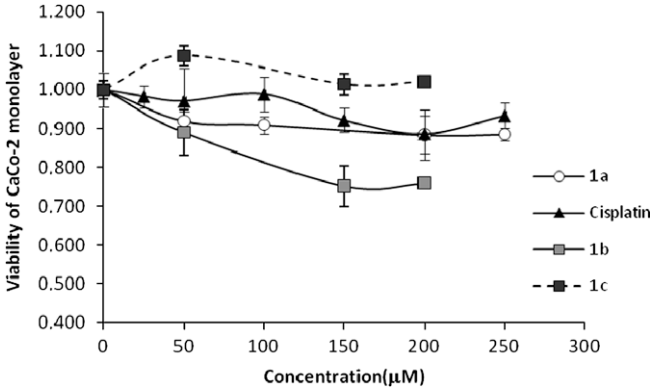


Figure 1. Toxicity of cisplatin and Rh_2(*L*-PheAla derivative)$_2$(OAc)$_2$ **1** on CaCo-2 cell monolayer. Cells were incubated with the compounds for a period of 4 h. Viability was assessed by the MTT reagent. Experimental points are the average of three repliques and the error bars are the standard deviation obtained with all the experimental points.

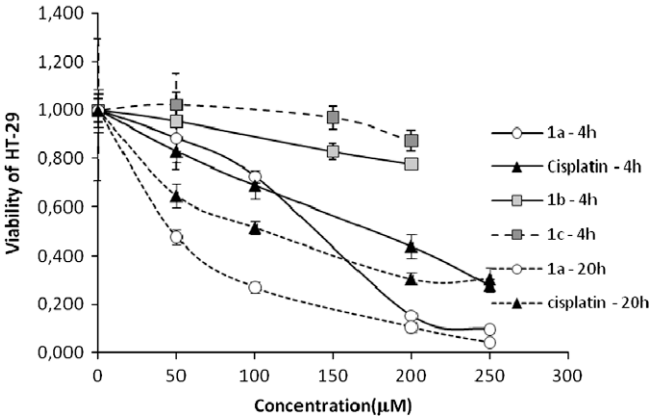


Figure 2. Anti-proliferative activity of cisplatin and Rh_2(*L*-PheAla derivative)$_2$(OAc)$_2$ **1** on HT-29 cells. Cells were exposed to the compounds for a period of both 4 and 20 h. Viability was assessed by the MTT reagent. Experimental points are the average of three repliques obtained in one experiment and the average of four repliques obtained in two independent experiments with cells from different flasks (eight repliques in total), in the case of 4 and 20 h incubation, respectively. The error bars are the standard deviation obtained with all the experimental points.

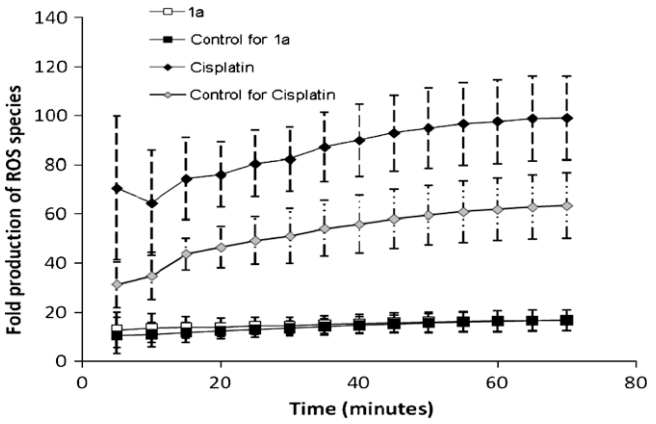


Figure 3. Intracellular reactive oxygen species produced in HT-29 cells exposed to cisplatin and Rh_2(*L*-PheAla)$_2$(OAc)$_2$ **1a**. HT-29 cells were incubated with 100 μM of each compound for 2 h. Control cells were incubated with the carrier solvent. Cells were washed and incubated with the probe dichlorofluorescein diacetate (DCF-DA) for determination of ROS. Excitation and emission wavelengths were 485 ± 20 nm and 528 ± 20 nm, respectively. Fluorescence detected at time t_1 (Ft_1) was substrated by the fluorescence at t_0 (Ft_0) and divided by (Ft_1–Ft_0) of the sample with the same pre-treatment but without incubation with the probe. Each experimental point corresponds to six repliques × 2 from two independent 96-well plates and the error bars to the standard deviation.

To assess if complex **1a** or cisplatin were likely inducing cell killing by apoptosis, we looked at caspase-3 activity and the obtained data suggest that cisplatin induced caspase-3 activation, whereas complex **1a** did not (Fig. 4). This result correlates with the increment of ROS in cisplatin-treated cells since oxidative stress is described as an upstream event of apoptosis.[16]

Complex **1a** was seen to inhibit growth of HT-29 cells leading eventually to cell death. Cells exposed to the highest doses of **1a** were clearly ill when examined in the microscope. Nevertheless, it was not detected any activation of caspase-3 after exposure to the dirhodium complex. (Fig. 3). But, for an exposure time of 20 h and a concentration of 100 μM, complex **1a** induced a greater decay in HT-29 cellular viability than cisplatin (Fig. 2). 100 μM cisplatin generated a significantly increase in reactive species, which did not take place with complex **1a**, thus suggesting different modes of action. Oxidative stress is possibly triggered by DNA

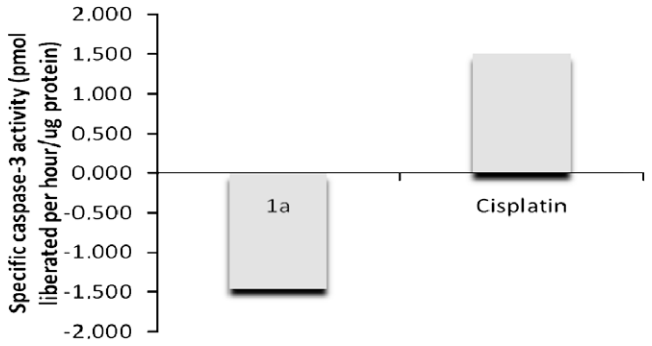


Figure 4. Assessment of specific caspase-3 activation in HT-29 cells exposed to cisplatin and $Rh_2(L\text{-PheAla})_2(OAc)_2$ **1a**. HT-29 cells were incubated with the compounds at 100 μM and for 20 h. In parallel, cells were simultaneously treated with complexes and an inhibitor of caspase-3 activity (supplied by Kit). Specific caspase-3 activity was assessed and calculated following the supplier's instructions. Due to the natural cell death in the test populations, the difference in absorbance (obtained in the absence and presence of inhibitor) can be negative and is considered null. Each condition was repeated three times.

damage[16] since published data supports formation of DNA adducts in the presence of cisplatin.[1,2] Despite $Rh_2(OAc)_4$ being described to interact with DNA,[8,9] complex **1a** might be inducing other responses as well that are likely dictating the cell fate. Indeed, structural constrains play a very important role as seen for the studied complexes **1b** and **1c**. They were not active and the only structural differences with complex **1a** are the presence of methoxy groups on the aromatic rings (Scheme 1).

Unless induction of apoptosis by complex **1a** needs a longer period than 20 h, as a result of a different cellular effect from cisplatin, complex **1a** may be causing necrosis. This type of cell death follows a different pattern and involves destruction of cellular membrane, which can be caused by perturbation of the cell osmotic equilibrium or energy deficiency.[7,8,16a,17] This form of cell killing has been reported to occur in the same cell model, in the presence of some natural compounds and extracts.[18] Further studies of this new compound should be encouraged for the development of new alternatives for colorectal cancer treatment.

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Supplementary data

Supplementary data (crystallographic data (excluding structure factors) for the structures in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 765710 and 765711. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44 (0)1223 336033 or e-mail: deposit@ccdc.cam.ac.uk]. Experimental procedures and spectroscopic data) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.04.002.

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